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An assessment of the fatty acid composition of horse-meat available at the retail level in northern Spain

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ABSTRACT

The objective of the present study was to assess the fatty acid composition of horse-meat available at the retail market in northern Spain. Horse steaks (*Longissimus thoracis et lumborum* muscle; $n = 82$) were purchased from butcher-shops and large grocery stores throughout six northern regions of Spain in two different seasons. Fat content differed significantly among regions (1.12 to 2.77%). Samples with higher intramuscular fat content presented the highest percentages of total monounsaturated fatty acids and the lowest contents of dimethylacetal and polyunsaturated fatty acids (PUFA), while the opposite was found in the leanest samples. A high variability was observed in the muscle and subcutaneous n-3 PUFA content. Overall, total n-3 PUFA content ranged between 1.17% and 18.9% in muscle fat and between 1.52% and 27.9% in backfat. Interestingly, almost 5% of surveyed loins from horse carcasses (4 out of 82) contained over 300 mg of linolenic acid per 100 g of meat which could have been marketed as a "source" of n-3 FAs according to Commission Regulation (EU) No 116/2010.

1. Introduction

Horses, as non-ruminant herbivores and hindgut fermenters, are adapted to ingest large quantities of forage in a continuous fashion. Dietary lipids are primarily digested by endogenous enzymes and 90–95% of total digested material is absorbed in the small intestine (Santos, Rodriguez, Bessa, Ferreira, & Martin-Rosset, 2011). Generally, the fatty acids (FA) are absorbed before being submitted to microbial metabolism occurring in the hindgut (cecum and colon), and therefore, biohydrogenation activity is low compared to ruminants. The digestive physiology of equines has been associated with high deposition of polyunsaturated fatty acids (PUFA) (Clauss, Grum, & Hatt, 2009) along with very low levels of less investigated *trans*-FAs (TFA) (Hartam, Shorland, & Moir, 1956) and conjugated linoleic acids (CLA) (Cicognini, Rossi, Sigolo, Gallo, & Prandini, 2014). Moreover, none of the scientific literature reviewed described the branched-chain FAs (BCFA) in horse tissues, even though Santos et al. (2011) observed considerable amounts of these FAs in the equine hindgut bacterial biomass.

In the nineteen-fifties, Shorland, Bruce, and Jessop (1952) already confirmed high levels of linolenic acid (LNA; 17%) and moderate levels of linoleic acid (LA; 4%) in grass-fed horse adipose tissue. In recent years, a higher accumulation of LNA was reported in horses managed under extensive grazing (23.9–24.3%) compared to animals finished with concentrates (0.90–8.90%; Guil-Guerrero, Rincón-Cervera, Venegas-Venegas, Ramos-Bueno, & Suárez-Medina, 2013; Lorenzo, Fuciños, Purriños, & Franco, 2010). Guil-Guerrero et al. (2013) even suggested that horse backfat could be a relevant source of energy and n-3 PUFAs for humans in unique environments. Horse-meat has been recently described as "healthier" in terms of its high n-3 PUFA content, it's almost absence of TFA, and its significantly lower footprint of greenhouse gas emission compared to ruminants (Belaunzaran et al., 2015; Lorenzo, 2013). In spite of its advantageous and nutritional qualities, horse-meat consumption is still scarce in many countries because of socio-cultural perceptions and the reoccurring mislabeling scandals. The objective of the present study was to detail the chemical and FA composition of horse muscle and adipose tissues available at the retail level in northern Spain, where equine production has become an interesting option in less favored mountain areas where human population is very low. Special emphasis was put on the PUFA content and other minor and less studied TFA, BCFA and CLA. Such data are relevant to provide "farm to fork" stakeholders with accurate information about the nutritional characteristics, and to improve consumer's perception and acceptance of this undervalued but edible product.

2. Material and methods

2.1. Sample collection

Horse steaks ($n = 82$) were purchased from butcher-shops (90%) and large grocery stores (10%) throughout six northern regions of Spain

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localized at both sides of the Cantabrian Mountains (Basque, BA; Navarre, NA; Cantabria, CA; Asturias, AS; Galicia, GA; Castile & Leon, CL); see map indicating sampling locations (Fig. 1). In these regions, horse-meat may be produced locally from animals grazed in valley or mountain areas and occasionally supplemented with moderate amounts of concentrates or imported, which is more likely when sold in large grocery stores. According to the limited information obtained from butchers and labels, most samples were obtained from animals that were managed and slaughtered locally. From 4 to 10 steaks per region were collected in two different seasons (spring and winter); from April to May of 2013 ($n = 41$) and from December of 2013 to January of 2014 ($n = 41$). The unequal number of samples per region were due to the fact that samples from three grocery chains were collected in the Basque and one in Asturias region. Horse-meat from grocery chains were not sampled in the other regions because the meat came from the same supplier. The two seasonal collections were initiated because of possible variations in meat composition related to different management practices linked to the time of year. Horse-meat collected in spring would likely come from animals that have entered the feedlot in the fall, while horse-meat collected in winter would likely come from animals that have been breastfed and grazed in mountain areas from spring to late autumn. However, precise details of production systems for most of the horse samples collected are unknown, and the information provided on labels or obtained from retailers was limited, but recorded in order to provide a possible explanation for the findings. Samples were transported to the laboratory located in the Lascaray Research Center (Vitoria-Gasteiz, Spain) in refrigerated coolers. Then, subcutaneous (SC) fat and *Longissimus thoracis et lumborum* (LTL) muscle were separated, vacuum packed and frozen at −80 °C for further analysis.

2.2. Chemical composition of muscle

Samples of LTL muscle were analyzed as described in Aldai, Lavín, Kramer, Jaroso, and Mantecón (2012b). Standard procedures were used for dry matter (ISO, 1999), crude protein (ISO, 2005), ether extract (AOCS, 2008) and ash (ISO, 2002) determinations.

2.3. Fatty acid analysis of adipose and muscle tissues

Seventy four SC fat samples were analyzed since 8 steaks were virtually devoid of external fat (butchers sometimes tend to remove the fat covering the rib joint). Fat samples were weighted (50 ± 1 mg), freeze-dried and directly methylated with sodium methoxide (Methanolic-Base, 0.5 N; Supelco). From the muscle tissue, lipids were extracted from 1.5 g of freeze-dried sample using chloroform-methanol (2:1, v/v; Folch, Lees, & Sloane-Stanley, 1957). Lipid aliquots (10 mg) from each steak were then separately methylated using base (Methanolic-Base, 0.5 N; Supelco) and acid (Methanolic HCl, 3 N; Supelco) methylations to ensure complete derivatization of total lipids and avoid CLA isomerization (Aldai et al., 2012a; Kramer et al., 1998; Kramer, Hernandez, Cruz-Hernandez, Kraft, & Dugan, 2008). For quantitative purposes, 1 mL of internal standard (IS; mixture of 0.5 mg/mL of 13:0 and 0.5 mg/mL of 23:0 methyl esters from Nu-Chek Prep Inc., Elysian, MN, USA) was added prior to methylation. Fatty acid methyl esters (FAME) were analyzed using a gas chromatograph equipped with a flame ionization detector (GC/FID; Agilent Technologies, Model 7890A, Wilmington, DE, USA) and automatic injector (Agilent Technologies, Model 7693). Samples were injected using a 50:1 split ratio, a Supelco SP2560 capillary column (100 m × 0.25 mm I.D., 0.2 μm coating, Bellefonte, PA, USA), and temperature programs previously described by Kramer et al. (2008). To resolve CLA isomers and some other overlapping FAMEs, samples were subjected to a second GC/FID analysis using a SLB-IL111 ionic liquid stationary phase column (100 m × 0.25 mm I.D., 0.2 μm coating; Supelco, Bellefonte, PA, USA) using a temperature program described by Delmonte et al. (2011). In both GC runs, hydrogen was used as a carrier gas at a flow rate of 1 mL/min, the injection volume was 1 μL, and injector and detector ports were set at 250 °C. For identification purposes, #463 and #603, individual FAME (21:0, 23:0, 26:0,

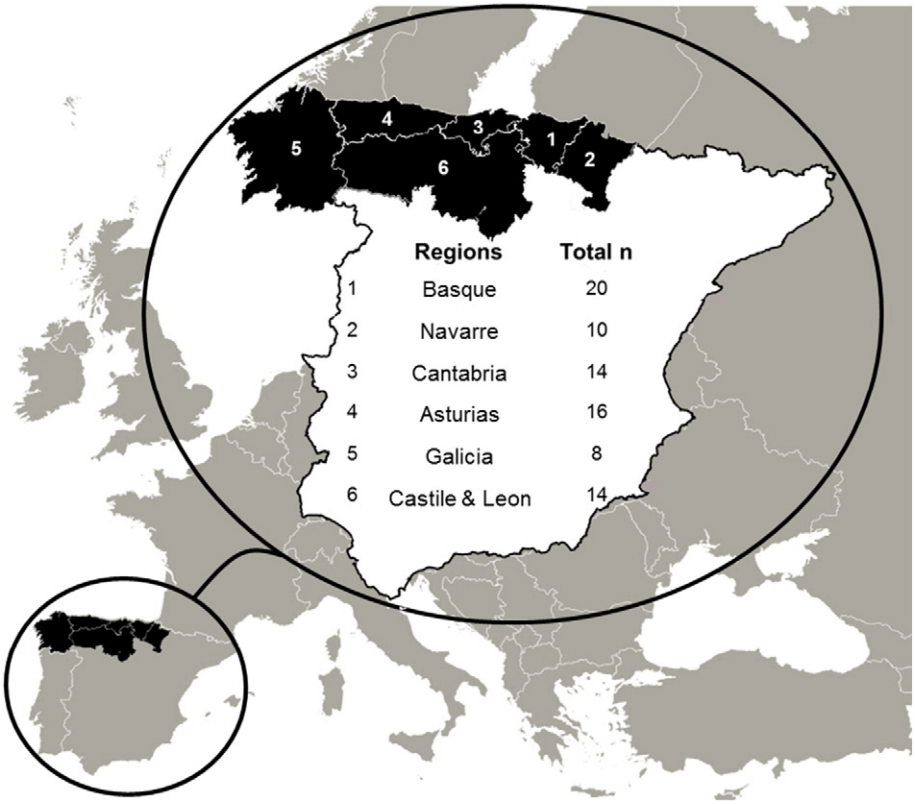

Fig. 1. A map showing sampling regions and the total number of samples collected in northern Spain.